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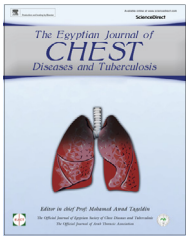

ORIGINAL ARTICLE

Role of epidermal growth factor receptor in malignant pleural mesothelioma and its value for successful chemical pleurodesis

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KEYWORDS

Malignant pleural mesothelioma;
Epidermal growth factor receptor;
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Abstract *Background:* The most common primary malignant tumor of the pleura is malignant mesothelioma. It is a highly aggressive tumor that has become a very important issue over recent years. Evidence suggests that EGFR is involved in the pathogenesis and progression of different carcinomas.

Aim of the work: To study the role of EGFR in MPM and to investigate its value for successful chemical pleurodesis.

Patients and Methods: This study included 53 patients with exudative pleural effusion. All were subjected to full history taking, clinical examination, CT chest, pleural biopsy histopathological analysis and EGFR Ab immunostaining. According to pleural biopsy histopathology, the patient population was divided into 3 subgroups; subgroup I (19 patients diagnosed benign pleural effusion); subgroup II (21 patients diagnosed MPM) and subgroup III (13 patients diagnosed malignant pleural effusion other than MPM).

Results: Regarding comparison between the 3 subgroups in the demographic data, there was no statistically significant difference in age, sex and smoking prevalence. Regarding pleural fluid analysis, there was no statistically significant difference in protein and LDH levels but there was

Abbreviations: EGFR, epidermal growth factor receptor; CT, computed tomography; MPM, malignant pleural mesothelioma; Ab, antibody; LDH, lactate dehydrogenase; SD, standard deviation; USA, United States of America; BTS, British Thoracic Society; dl, decilitre; IU, international unit; ErbB, erythroblastosis oncogene B; NSCLC, non small cell lung cancer; TKI, tyrosine kinase inhibitors

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statistical significance in sugar levels between subgroups I and II. There was statistical significance regarding predominant cell pattern during pleural fluid cytology. Also there was statistical significance regarding immunostaining for the detection of EGFR in pleural biopsy among study subgroups. However, there was no statistical significance regarding comparison between success of chemical pleurodesis and expression of EGFR among malignant subgroups of pleural effusion.

Conclusion: There is evidence that EGFR is frequently overexpressed in MPM and therefore may be used as a potential therapeutic target.

Introduction

The most common primary malignant tumor of the pleura is malignant mesothelioma. It arises from mesothelial surfaces of the pleural and peritoneal cavities, as well as from the tunica vaginalis and pericardium [1].

Malignant pleural mesothelioma is a highly aggressive tumor that has become a very important issue over recent years [2].

Epidermal growth factor receptor exists on the cell surface and is activated by binding of its specific ligands, including epidermal growth factor and others [3]. The resulting signaling network initiates diverse cellular pathways leading to proliferation, migration, gene transcription, cell cycle progression and cell survival [4].

Evidence suggests that the EGFR is involved in the pathogenesis and progression of different carcinoma types. In vivo and in vitro studies have shown that these proteins are able to induce cell transformation [5].

Subjects

The present study included fifty three patients who were selected from the Chest Department inpatients, Kasr Alainy Hospital. The selected patients had either exudative pleural effusion according to the light's criteria [6], pleural thickening or pleural masses that allows pleural biopsy to be performed. Patients with transudative pleural effusion, bleeding disorders or unfit for pleural biopsy procedures were excluded from the study.

The included patients were divided into 3 subgroups according to histopathological examination of the pleural biopsy:

- Group I: included 19 patients with benign pleural effusion.
- Group II: included 21 with MPM.
- Group III: included 13 patients with malignant pleural effusions other than mesothelioma.

Methods

All included patients were subjected to written informed consent, full history taking, detailed clinical examination, plain chest X-ray, CT chest, and thoracentesis with chemical and cytological analysis of the pleural fluid samples. Pleural biopsy was also obtained and sent for histopathological examination to reach a final diagnosis and to search for the presence of mesothelial cells in pleural biopsy. Then immunohistochemical staining was done in the pleural biopsy specimens that showed the presence of mesothelial cells for the detection of epidermal growth factor receptor.

Finally chemical pleurodesis was performed for malignant cases only, when the pleural fluid drainage was less than 100 cc/day and the lung was clinically and radiologically fully expanded.

Out of the 53 patients who formed the study population, 48 cases were diagnosed by medical thoracoscopic pleural biopsy, one case was diagnosed by sonar guided pleural biopsy and another one was diagnosed by Abram's needle. Also 3 patients underwent open thoracotomy and decortication.

Histopathological examination of the pleural biopsy

All tissue samples were routinely processed, fixed in 10% buffered formalin, dehydrated, cleared and embedded in paraffin wax according to the routine processing procedure. Two sections (5 microns thick) were prepared from each tissue paraffin block. One was stained by Hematoxylin and Eosin (H&E) staining for routine histopathologic examination and the other sections were on charged slides and subjected to immunohistochemical staining by mouse anti-EGFR.

Then all patients were classified according to the results of the routine histopathological analysis of the pleural biopsy into 3 subgroups as mentioned previously.

Immunohistochemical staining for detection of EGFR in pleural biopsy

Immunohistochemical staining by labeled streptavidin–biotin method of immunohistochemistry for EGFR by mouse anti-EGFR clone 31G7, antibody conc. 357 μg/ml serial number 20718528L, manufacturer: Genemed biotech USA, REF: 61-0027-2 and dilution of 1:50–1:100 for 30–60 min at room temperature).

After deparaffinization and rehydration, sections were placed in 3% hydrogen peroxide for 20 min to inactivate endogenous peroxidase and treated by microwave at 121 °C in citrate buffer (10 mM, pH 6.0) for 10 min as an antigen retrieval method. After cooling to room temperature for 30 min, specimens were non-specifically blocked by incubation with normal rabbit serum for 15 min at room temperature. Sections were incubated with the primary antibodies for one hour at room temperature. The sections were then subjected to a three-step labeling procedure, with the use of streptavidin biotin complex using 3,3′-diaminobenzidine as the chromogen and the sections were faintly counterstained with Hematoxylin.

The positive control for EGFR consisted of sections from metaplastic carcinoma of the breast known as positive for

EGFR. Sections of the positive control were used in each run. Pleural biopsies that exhibited either cytoplasmic or membranous immunoreactivity in mesothelial cells are considered as positive [7].

Statistical analysis

Data were statistically described in terms of mean ± standard deviation (±SD), median and range or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student *t* test for independent samples in comparing 2 groups when normally distributed and the Mann Whitney *U* test for independent samples when not normally distributed. Comparison of numerical variables between more than two groups was done using a one way analysis of variance (ANOVA) test with posthoc multiple 2-group comparisons in normal data and the Kruskal Wallis test with posthoc multiple 2-group comparisons in non-normal data. For comparing categorical data, the Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. *P* values less than 0.05 were considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

The present study included 53 patients who fulfilled the selection criteria and formed the study population. According to histopathological analysis of pleural biopsy specimens, the study population was divided into 3 subgroups as previously mentioned. There was no statistically significant difference between the 3 study subgroups in the patients' characteristics (Table 1).

Regarding pleural fluid chemical analysis, there was no statistically significant difference between the study subgroups in protein and LDH levels, however there was statistically significant difference in the sugar level between subgroups I (benign pleural effusion) and II (MPM) (Table 2). There was also statistically significant difference regarding the predominant cell pattern in the pleural fluid among the study subgroups. In subgroup I, the predominant cell was neutrophil, while lymphocyte was the predominant cell in subgroups II and III. Malignant cells were found only in 2 patients among subgroup II (Fig. 1).

After histopathological analysis of the pleural biopsy specimen, 11 out of the 19 patients of subgroup I were diagnosed as chronic nonspecific pleurisy (57.9%), 4 patients were empyema (21.1%), 1 patient was sarcoidosis (5.3%), 1 patient was systemic lupus (5.3%) and another 2 patients were diagnosed as tuberculous pleurisy (10.5%). subgroup III (13 patients) which is the subgroup of malignant pleural effusions other than mesothelioma included 2 patients diagnosed as lymphoma (15.4%), 4 cases diagnosed as metastatic adenocarcinoma of breast origin (30.8%) and another 7 patients were diagnosed as metastatic adenocarcinoma of lung origin (53.8%) (Figs. 2 and 3).

As regards the presence of mesothelial cells in the pleural biopsy specimens among the study subgroups; mesothelial cells were present only in 8 out of the 19 patients (42.1%) of subgroup I, while it was present in 19 (90.5%) out of the 21 patients of subgroup II and they were present in all patients (13 patients) of subgroup III. The difference between the study subgroups was found to be statistically significant with a *P*-value of 0.000 (Fig. 4).

Imunnohistochemical staining for the detection of EGFR was only done in the pleural biopsy specimens that showed the presence of mesothelial cells during routine histopathological analysis. Among subgroup I, 8 out of the 19 patients were

Table 1 Patient characteristics.

	Study subgroups			Total (n = 53)	P-value
	Subgroup (I) (n = 19)	Subgroup (II) (n = 21)	Subgroup (III) (n = 13)		
Sex Distribution (count, % within subgroup)					
Female	8 (42.1%)	13 (61.9%)	7 (53.8%)	28 (52.8%)	0.455
Male	11 (57.9%)	8 (38.1%)	6 (46.2%)	25 (47.2%)	
Age (mean ± SD) (years)	46.53 ± 14.20	55.10 ± 14.68	56.46 ± 18.02	52.36 ± 15.73	0.126
Smoking prevalence (count, % within subgroup)					
Smoker	9 (47.4%)	8 (38.1%)	5 (38.5%)	22 (41.5%)	0.811
Non smoker	10 (52.6%)	13 (61.9%)	8 (61.5%)	31 (58.5%)	

Table 2 Chemical analysis of the pleural fluid among study subgroups.

	Study subgroups			Total (n = 53) Mean ± S.D	*P*-value
	Subgroup (I) (n = 19) Mean ± S.D	Subgroup (II) (n = 21) Mean ± S.D	Subgroup (III) (n = 13) Mean ± S.D		
Protein (g/dl)	4.68 ± 1.16	4.79 ± 1.25	4.52 ± 0.93	4.69 ± 1.13	0.804
LDH (IU/L)	599.21 ± 496.13	647.30 ± 427.59	581.38 ± 541.84	613.25 ± 474.19	0.918
Sugar (g/dl)	70.37 ± 28.98	93.19 ± 28.72	93.38 ± 22.87	85.06 ± 29.18	0.020*

* *P*-value <0.05 = statistically significant.

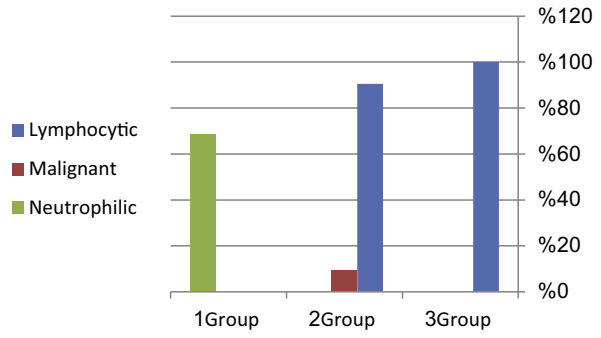

Figure 1 The predominant cell pattern during pleural fluid cytological analysis among the study subgroups.

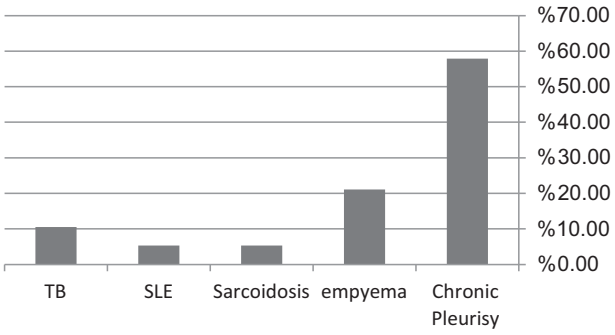

Figure 2 Histopathological subtypes among subgroup I (Benign pleural effusion) of the study population.

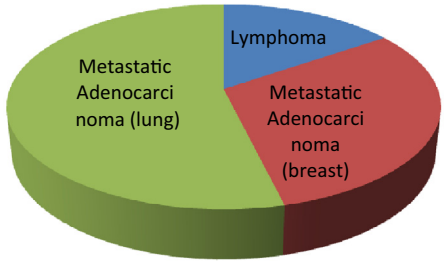

Figure 3 Histopathological subtypes among subgroup III (Malignant pleural effusions other than mesothelioma) of the study population.

only stained due to lack of mesothelial cells in remaining 11 patients. All 8 cases were positive for immunostaining by anti-EGFR antibody. Among subgroup II, 19 out of the 21 patients were stained for the detection of EGFR. 14 (73.7%) out of these 19 patients were stained positive (Fig. 5) while the remaining 5 patients (26.3%) were stained negative. All the 13 patients of subgroup III were stained for detection of EGFR. In this subgroup only 6 (46.2%) out of the 13 patients were stained positive while the remaining 7 patients (53.8%) were stained negative. There was a statistically significant difference as regards the comparison between the three study subgroups in the immunohistochemical staining for the detection of EGFR with a *P*-value of 0.029 (Table 3).

Chemical pleurodesis was done only for patients with malignant pleural effusion, either MPM (subgroup II) or

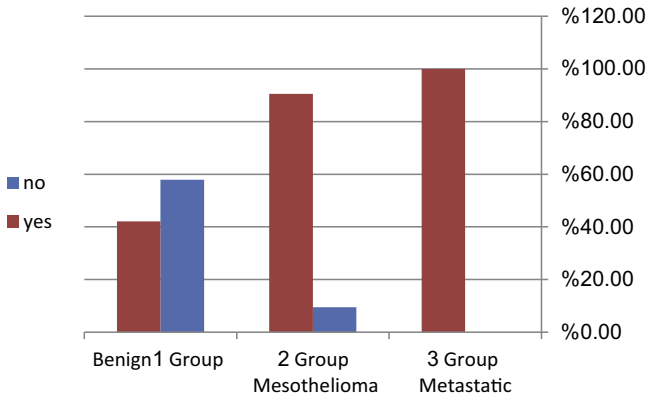

Figure 4 Presence of mesothelial cells in the pleural biopsy specimens among the study subgroups.

malignant pleural effusions other than mesothelioma (subgroup III). Chemical pleurodesis was done for 20 out of the 21 patients forming subgroup II, because the remaining patients did not have effusion but only pleural thickening. Among patients who underwent chemical pleurodesis in subgroup II, 19 cases (95%) succeeded and only 1 case (5%) failed. Chemical pleurodesis was done to all the 13 patients of subgroup III, among them 9 cases (69.2%) succeeded and 4 cases (30.8%) failed. There was no statistically significant difference in the success rate of chemical pleurodesis between subgroups II and III (Fig. 6).

As regards the relationship between positivity of EGFR immunostaining and characteristics of the study subgroups, it was found that there was no statistical significance regarding the comparison between age, sex, smoking prevalence, biochemical analysis of pleural fluid, histopathology of the pleural biopsy within each subgroup and EGFR immunostaining.

Also, there was no statistically significant difference between pleurodesis success rate and EGFR immunostaining in the malignant subgroups of the study population (Table 4).

Discussion

Malignant mesothelioma is an insidious neoplasm with a dismal prognosis arising from the mesothelial surfaces of the pleural and peritoneal cavities, as well as from the tunica vaginalis and pericardium. 80% of all cases of mesothelioma are pleural in origin [1]. Malignant pleural mesothelioma is a highly aggressive tumor that was previously considered to be rare and has become a very important issue over recent years [2].

Members of the EGFR family have frequently been implicated in various forms of human cancers and serve both as prognostic markers and as therapeutic targets. Several phenomena are responsible for abnormal activation of these receptors in tumors, including overexpression, amplification and constitutive activation of mutant receptors or autocrine growth factor loops [4].

Clinical developments over the past decade have identified several novel therapeutic agents which inhibit tyrosine kinase activity, either by direct receptor inhibition or indirect inhibition of tyrosine kinase controlled pathways [8].

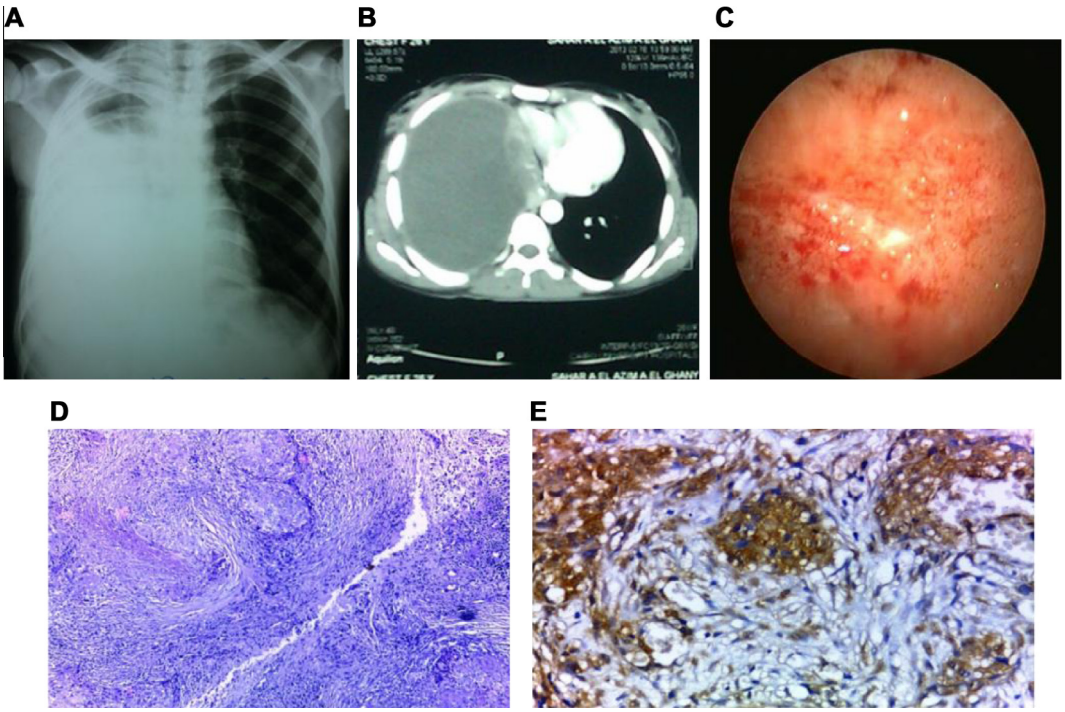

Figure 5 Female patient 36 years old, presented by gradual progressive dypsnea of 3 months duration. (A) Plain chest X-ray showing massive right side pleural effusion. (B) Axial cut of CT chest showing pleural effusion with thickening of costal and mediastinal pleura. (C) Thoracoscopic image showing diffuse thickening of costal pleura with some nodulation. (D) Microscopic image showing epithelial type mesothelioma (H&Ex200). (E) Microscopic image showing EGFR positive staining (immunoperoxidase, DAB × 200).

Table 3 Immunohistochemical staining for detection of EGFR in the pleural biopsy among study subgroups.

			Study subgroups			Total
			Subgroup (I)	Subgroup (II)	Subgroup (III)	
Immunostaining	−Ve	Count	0	5	7	12
		% within Group	0.0%	26.3%	53.8%	30.0%
	+Ve	Count	8	14	6	28
		% within Group	100.0%	73.7%	46.2%	70.0%
Total		Count	8	19	13	40
		% within Group	100.0%	100.0%	100.0%	100.0%
P-value = 0.029*						

* *P*-value < 0.05 = statistically significant.

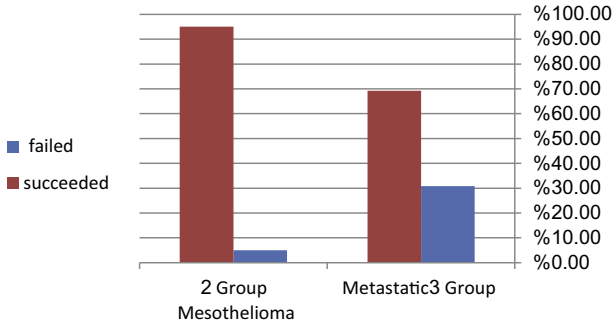

Figure 6 Statistical comparison of the success rate of chemical pleurodesis between subgroups II and III of the study population.

The aim of the current study was to evaluate the role of epidermal growth factor receptor in malignant pleural mesothelioma and to investigate its value for successful chemical pleurodesis.

As regards the sex distribution in the current study, subgroup I (patients with benign pleural effusion) included 8 females (42.1%) and 11 males (57.9%), subgroup II (MPM) included 13 females (61.9%) and 8 males (38.1%) and subgroup III (patients with malignant pleural effusion other than MPM) included 7 females (53.8%) and 6 males (46.2%). There was no statistically significant difference as regards sex distribution between the benign and malignant subgroups of pleural effusion. Also there was no statistically significant difference between the male and female distribution within each subgroup of the study population. All subgroups were sex matched with a *P*-value of 0.455.

Table 4 Relationship between success rate of chemical pleurodesis and results of EGFR immunostaining among subgroup II and III of the study population.

			EGFR immunostaining					
			Subgroup II			Subgroup III		
			−Ve	+Ve	Total	−Ve	+Ve	Total
Pleurodesis	Failed	Count	0	1	1	3	1	4
		% within IS	0.0%	7.7%	5.6%	42.9%	16.7%	30.8%
	Succeeded	Count	5	12	17	4	5	9
		% within IS	100.0%	92.3%	94.4%	57.1%	83.3%	69.2%
Total	Count	5	13	18	7	6	13	
	% within IS	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	
P-value	1.000	0. 559						

The current study agreed with Martínez-Moragón et al. [9] who studied malignant pleural effusion regarding prognostic factors for survival and response to chemical pleurodesis in a series of 120 cases. They found in their study population that 55% were females and 45% were males. Also Burgers et al. [10] study about implementation of guidelines in four hospitals for pleural drainage and pleurodesis found that females were 60% and males 40%. Also Ismail [11], studied the role of vascular endothelial growth factor in the diagnosis of pleural effusions of different origins and he found that males were 52.9% in the group with infectious pleural effusion.

On the other hand Akl et al. [12], a ten-year (1998–2007) multicenter study about the epidemiology of mesothelioma in Egypt, found that male/female ratio was 1.35/1. Similarly Reid et al. [13] studied the age and sex differences in malignant mesothelioma after residential exposure to blue asbestos (crocidolite) and stated that the rate is higher in male subjects than females.

As regards the mean age in the current study population, in subgroup I, the mean age was 46.53 years, in group II, it was 55.10 years and in group III, it was 56.46 years. There was no statistical significance regarding the difference in mean age among the study subgroups with *P*-value 0.126.

This study agreed with Ismail [11] who found that the mean age in infectious pleural effusion group was 49.8 years and with Akl et al. [12] who found that the mean age in mesothelioma patients was 50.78 years. This is consistent with the fact given the latency period, which is 20 to more than 40 years moderate asbestos exposure would probably carry risk for the asbestos worker at the age of 55 years or older to develop mesothelioma [14]. Also Martínez-Moragón et al. [9] found that the mean age in patients with malignant pleural effusion was 60 years. Similarly Burgers et al. [10] found in his study that the mean age was 57 years.

As regards smoking prevalence among the study subgroups, subgroup I included 10 non smokers (52.6%) and 9 smokers (47.4%), subgroup II included 13 non smokers (61.9%) and 8 smokers (38.1%) and subgroup III included 8 non smokers (61.5%) and 5 smokers (38.5%). The difference in the smoking prevalence between the benign and malignant subgroups of pleural effusion was found to be statistically insignificant. Also the difference in the smoking prevalence within each subgroup of the study population was found to be statistically insignificant. All study subgroups were matched in the smoking prevalence with a *P*-value of 0.811.

The present study agreed with Muscat and Wynder [15] who studied cigarette smoking, asbestos exposure and malignant mesothelioma. They stated that there is no association between cigarette smoking and mesothelioma, while Soe et al. [16] who studied malignant pleural effusion found that 82.2% of cases were either heavy smokers or ex-smokers.

Regarding pleural fluid chemical analysis, in subgroup I, the mean values of total proteins, LDH and sugar were 4.68 g/dl, 599.21 IU/L and 70.37 mg/dl respectively, in subgroup II were 4.79 g/dl, 647.30 IU/L and 93.19 mg/dl, respectively and in subgroup III were 4.52 g/dl, 581.38 IU/L and 93.38 mg/dl, respectively. There was no statistical significance as regards the comparison between the 3 subgroups in the mean value of total proteins and LDH but there was statistically significant difference as regards the comparison between subgroups I and II in the mean value of sugar, being lower in subgroup I than subgroup II. This could be explained on the basis that subgroup I included 4 patients with empyema and 2 cases diagnosed as tuberculous pleural effusion.

In BTS guidelines for the investigation of a unilateral pleural effusion in adults Maskell and Butland [17] stated that the lowest glucose concentrations are found in empyema. Also Chernow and Sahn [18], who studied 96 patients for carcinomatous involvement of the pleura found that the mean value for total proteins was 3.7 g/dl and that for sugar was 120 mg/dl.

Also Gottehrer et al. [19] studied pleural fluid analysis in malignant pleural mesothelioma prognostic implications and found that the mean values for total protein, LDH and sugar were 4.3 g/dl, 516 IU/L and 75 mg/dl, respectively.

Regarding the predominant cell pattern during pleural fluid cytological analysis in the present study, there was statistically significant difference between the study subgroups with a *P*-value of 0.000. This agreed with Maskell and Butland [17] who found that polymorphonuclear cells predominate, when the patient has an acute process affecting the pleural surfaces.

Light [20] stated that the percentage of cases in which the cytologic study of the pleural fluid establishes the diagnosis of a malignant pleural effusion ranges from 40–87%. Also Antony et al. [21] study for management of malignant pleural effusions stated that the diagnostic yield of cytology for mesothelioma is 58%. This goes hand with hand with Ismail [11] who found that 40% of the malignant cases had a positive cytology for malignant cells.

According to the histopathological subtypes among subgroup I, the present study disagreed with Ismail [11] who found that 11 cases out of 28 (benign pleural effusion) were diagnosed as tuberculous pleural effusion while 17 cases were diagnosed as empyema.

Regarding histopathological subtypes among subgroup III of the study population, the present study agreed with Antony et al. [21] who stated that lung carcinoma has been the most common neoplasm, accounting for approximately 1/3 of all malignant effusions with 25–52% and breast carcinoma is the second most common with 3–27%. Lymphomas, including both Hodgkin's disease and non Hodgkin's lymphoma, are also an important cause of malignant pleural effusions with 12–22%.

According to the presence of mesothelial cells in the pleural biopsy specimens, the low percentage (42.1%) of mesothelial cells in the benign subgroup of pleural effusion (subgroup I) in comparison to the malignant subgroups (subgroup II; MPM and subgroup III) could be explained by the study of Herbert and Gallagher [22] about pleural biopsy in the diagnosis of malignant mesothelioma who found that in inflammatory conditions the mesothelial lining was usually replaced by granulation tissue. Also this agreed with Mutsaers [23] who studied the mesothelial cell and found that injury to the mesothelium triggers events leading to the migration of mesothelial cells from the edge of the lesion center and desquamation of cells into the serosal fluid.

Regarding immunohistochemical staining for detection of EGFR in the pleural biopsy among the study subgroups

Among subgroup I, 8 (100%) patients were stained positive for the detection of EGFR while among subgroup II, 14 (73.7%) out 19 patients were stained positive. This agreed with Ramael et al. [7] who studied immuno-histochemical distribution patterns of EGFR in malignant mesothelioma and non neoplastic mesothelium and they concluded that there is strong expression of EGFR in both malignant mesothelioma and in non neoplastic pleural mesothelium. Also Ikeda et al. [24] studied EGFR aberrations in malignant mesothelioma and found that the expression of EGFR was not different between malignant mesothelioma and non neoplastic mesothelial cells. Similarly Dazzi et al. [25] reported that 68% of mesothelioma specimens showed EGFR overexpression and Govindan et al. [26] showed EGFR overexpression in 11 of 19 mesothelioma specimens.

On the other hand Destro et al. [27] studied EGFR overexpression in malignant pleural mesothelioma and found that EGFR immunoreactivity was documented in 34/61 (55.7%) cases of malignant pleural mesothelioma.

Although in the present study, only 6 (46.2%) out of the 13 patients forming subgroup III were stained positive for EGFR, Koutsionasios et al. [28] who studied the value of expression of EGFR, telomerase and topoisomerase IIα in malignant effusion smears before and after chemotherapy found positive expression of EGFR in 69.5% of the cases.

Regarding evaluation of the success rate of chemical pleurodesis among subgroups II and III of the study population, the current study disagreed with Ak et al. [29] who evaluated pleurodesis in follow up and treatment of malignant pleural mesothelioma patients and found that pleurodesis succeeded in only 62% of the patients but agreed with Antony et al. [21] who stated that pleurodesis studies have demonstrated clinical success rates of 80–85%.

Regarding the relationship between positivity of EGFR immunostaining and some characteristics of the study subgroups

Among subgroup I, 8 patients were stained positive for EGFR. These 8 patients included 2 (25%) non smokers and 6 (75%) smokers. Among subgroup II (MPM), immunostaining positive cases (14 patients) included 9 (64.3%) non smokers and 5 (35.7%) smokers, while immunostaining negative cases (5 patients) included 3 (60%) non smokers and 2 (40%) smokers. This agreed with O'Donnell et al. [30] who studied the expression of ErbB receptors and mucins in the airways of long term current smokers and suggested that long term current smoking induces enhanced epidermal growth factor receptor expression in vivo and these increases are not associated with the presence of neutrophilic inflammation.

Among subgroup II, the number of immunostaining positive cases was 14 patients and included 9 (64.3%) females and 5 (35.7%) males, while the number of immunostaining negative cases was 5 patients which included 3 (60%) females and 2 (40%) males. The difference between immunostaining positive and negative cases as regards sex distribution was found to be statistically insignificant. This agreed with Rena et al. [31] who studied EGFR overexpression in malignant pleural mesothelioma: Prognostic correlations showed that there was no association recorded between EGFR positive staining and gender.

Also among Subgroup II, the mean age of immunostaining negative cases was 52 years, while the mean age of immunostaining positive cases was 54.57 years. The difference between immunostaining negative and immunostaining positive cases in the mean age was found to be statistically insignificant with a *P*-value of 0.547. This again agreed with Rena et al. [31] who found in his study no association recorded between EGFR positive staining and age.

Among subgroup III, the number of immunostaining positive cases was 6 patients and included 3 females (50%) and 3 males (50%), while the number of immunostaining negative cases were 7 patients including 4 females (57.1%) and 3 males (42.9%). The difference between immuno-staining positive and negative cases as regards sex distribution was found to be statistically insignificant with a *P*-value of 1.000. The current study disagreed with Hsieh et al. [32] who stated that female sex predicted the presence of EGFR mutations in lung adenocarcinomas, while Zheng et al. [33] stated that there was no correlation between EGFR and sex.

As regards the relationship between smoking prevalence and immunohistochemical staining for EGFR in subgroup III, the number of EGFR immunostaining positive cases was 6 patients and included 3 non smokers (50%) and 3 smokers (50%), while the number of immunostaining negative cases was 7 patients and included 5 non smokers (71.4%) and 2 smokers (28.6%). The difference between immunostaining positive and negative cases as regards smoking prevalence was found to be statistically insignificant with a *P*-value of 0.592. In contrast Zheng et al. [33] study which was small tumor size and limited smoking history predicts activated EGFR in early stage non small cell lung cancer (NSCLC), found an inverse correlation between EGFR and the degree of tobacco smoking and Kumar et al. [34] observed that EGFR expression was significantly higher in current smokers than in past smokers, who in turn had higher EGFR levels than those who never smoked and they suggested that smoking may contribute to increased EGFR expression, possibly through increased hypoxia in the tumor tissue of smokers.

In subgroup III, the number of immunostaining positive cases was 6 patients and all of them were diagnosed as metastatic adenocarcinoma but the number of immunostaining

negative cases were 7 patients and included 2 cases with lymphoma (28.6%) and 5 cases with metastatic adenocarcinoma (71.4%). The difference between positive and negative cases of immunostaining regarding the histopathology of the pleural biopsy was found to be statistically insignificant with a *P*-value of 0.155.

Although the literature concerning EGFR positive NSCLC and its response to TKI therapy is broad, the number of studies focusing on EGFR positivity in pleural involvement has been less robust [35]. The current study agreed with Shamblin et al. [36] who studied EGFR mutations in malignant pleural effusions from lung cancer and found that the incidence of EGFR positivity in malignant pleural effusion appears to range from 24–68.7%. Also this study agreed with Courville et al. [37] who studied pseudo-epitheliomatous hyperplasia in cutaneous T-cell lymphoma with particular interest in epithelial growth factor expression and stated that no expression of EGF could be detected in cutaneous and nodal B-cell lymphomas or in a normal lymph node.

Regarding the relationship of success rate of chemical pleurodesis and EGFR immunostaining

Among the 18 patients who underwent chemical pleurodesis in subgroup II, the number of immunostaining positive cases was 13 patients (12 succeeded [92.3%] and 1 failed [7.7%]), but the number of immunostaining negative cases was 5 patients and all of them had successful chemical pleurodesis. The comparison between immunostaining positive and negative cases in the success rate of chemical pleurodesis revealed no statistically significant difference (*P*-value = 1.000).

In subgroup III, 13 patients had undergone chemical pleurodesis. The number of immunostaining positive cases was 6 patients (5 succeeded [83.3%] and 1 failed [16.7%]), but the number of immunostaining negative cases was 7 patients (4 succeeded [57.1%] and 3 failed [42.9%]). As regards the comparison between positive and negative cases of immunostaining in the success rate of chemical pleurodesis, there was no statistically significant difference (*P*-value = 0.559).

In conclusion, this study found that epidermal growth factor receptor is frequently over expressed in malignant pleural mesothelioma samples and therefore may be used as a potential therapeutic target but there was no association between the success rate of chemical pleurodesis and expression of EGFR by the tumor either MPM or metastatic adenocarcinoma because there are many factors which may affect the success rate of chemical pleurodesis including the tumor burden. Further studies are needed to evaluate the prognostic value of EGFR mutation and overexpression in patients with MPM.

Conflict of interest

None.

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